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A STUDY OF SALIVA LUBRICATION USING A COMPLIANT ORAL MIMIC

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ABSTRACT

Due to ethical issues and the difficulty in obtaining biological tissues, it is important to find synthetic elastomers that can be used as replacement test media for research purposes. An important example of this is friction testing to understand the mechanisms behind mouthfeel attributes during food consumption (e.g. syrupy, body and clean finish), which requires an oral mimic. In order to assess the suitability of possible materials to mimic oral surfaces, a sliding contact is produced by loading and sliding a hemispherical silica pin against either a polydimethyl siloxane (PDMS), agarose, or porcine tongue sample. Friction is measured and elastohydrodynamic film thickness is calculated based on the elastic modulus of the samples, which is measured using an indentation method. Tests were performed with both saliva and pure water as the lubricating fluid and results compared to unlubricated conditions.

PDMS mimics the tongue well in terms of protein adhesion, with both samples showing significant reductions in friction when lubricated with saliva versus water, whereas agarose showed no difference between saliva and water lubricated conditions. This is attributed to PDMS's -O-Si(CH₃)₂- group which provides excellent adhesion for the saliva protein molecules, in contrast with the hydrated agarose surface. The measured modulus of the PDMS (2.2 MPa) is however significantly greater than that of tongue (3.5 kPa) and agarose (66-174 kPa). This affects both the surface (boundary) friction, at low sliding speeds, and the entrained elastohydrodynamic film thickness, at high speeds.

Utilising the transparent PDMS sample, we also use fluorescence microscopy to monitor the build-up and flow of dyed-tagged saliva proteins within the contact during sliding. Results confirm the lubricous boundary film forming nature of saliva proteins by showing a strong correlation between friction and average protein intensity signals (cross correlation coefficient = 0.87). This demonstrates a powerful method to study mouthfeel mechanisms.

KEYWORDS

Saliva, oral mimic, mouthfeel, friction, roughness, stiffness, lubrication, fluorescence microscopy.

1. INTRODUCTION

Due to ethical issues and the difficulty in obtaining biological tissues, it is necessary to find synthetic elastomers that can be used as replacement test media for research purposes. A key example of this is friction testing to understand the mechanisms behind mouthfeel attributes during food consumption (e.g. syrupy, body and clean finish), which requires an oral mimic. This is important since the acceptability of food and beverages depend critically on their mouthfeel, which results from tribological and rheological processes [1]. Moreover, a poor understanding of these processes currently limits the development of healthy formulations that can replicate foods while reducing ingredients such as fat [2], [3]. When mimicking the oral mucosa for these in vitro tribological studies of foods and beverages, consideration must be made of the mucosal pellicle. Like the acquired enamel pellicle on teeth, this is a subset of salivary proteins that specifically bind to oral epithelial cells [4]. Unlike the acquired enamel pellicle, the mucosal pellicle is mostly composed of mucins and secretory IgA. This layer is driven by the interaction of salivary mucins (muc5b and muc7) with membrane-bound mucin (muc1) expressed on oral epithelial cells [5]. Mucins are large highly glycosylated proteins which retain considerable amounts of water when initially secreted [6]. Thus, in addition to saliva lubricating the surface, there is also a hydrogel-like layer adjacent to the surface. All too frequently however, saliva is omitted from in vitro tests as it was cited as being too inconvenient to collect in sufficient quantities or considered too complex to give consistent results.

Previously, the oral mucosa was mimicked using glass or other hard substrates [7]. More recently elastic substrates have been used which introduced soft-tribology with Hertzian mechanics. In an important investigation by Dresselhuis et al. [8], the surface characteristics of pig tongue were compared with those of PDMS. Their investigation concluded that PDMS showed dissimilarities in surface characteristics to those of a tongue surface, since the oral mucosa and PDMS rubbers, even with a structured surface to reproduce biological scenarios, were not interchangeable in tribological experiments. However, this widely cited paper has a critical shortcoming in that it used only emulsion as the lubricant and saliva interactions were completely ignored. Other work carried out on biological surfaces, but without the presence of saliva include

studies by Adams et al. [9] and Tang et al. [10], [11] into the lubricating properties of human skin. Adams et al. used a smooth glass or polypropylene, spherical tipped probe sliding against a human forearm, while Tang et al. employed shaved porcine skin. Results were reported for a range of lubricating conditions, but repeatability of testing was difficult to achieve. Prinz et al. [12] did investigate the frictional properties between two pig mucosal surfaces lubricated with human saliva. However, scant data is presented and no comparison is made between different component materials.

For the majority of research, crosslinked polydimethyl siloxane (PDMS) has been chosen because of its elastic properties, easy handling and relatively low stiffness, comparable to soft biological tissues [13][14]. PDMS is utilized as one [15],[16],[10],[11] or both [17],[18],[19] of the contacting surfaces in the tribological contact to maintain low contact pressures and create the conditions for isoviscous-elastohydrodynamic lubrication(I-EHL) to occur. One key advantage of PDMS which has contributed to its widespread use is its ease of fabrication. Prior to crosslinking, PDMS can be cast into suitable moulds of almost any desired shape. Other attractive features of PDMS include its physiological inertness, availability, low unit cost, as well as its good thermal and oxidative stability.

PDMS is a transparent silicon-based organic polymer, used to represent biological materials in numerous tribological studies (e.g. [19] [8], [20]). It is highly compliant, with a Young's modulus $E \approx 0.57$ to 3.7 MPa (depending on degree of crosslinking) [21], due to its uniquely low glass transition temperature ($T_g \approx -125$ °C) [22]. The surface of PDMS is hydrophobic, due to its repeating $-\text{O}-\text{Si}(\text{CH}_3)_2-$ group [9] but can be made hydrophilic by plasma cleaning. In addition to this, PDMS is being used extensively in polymeric microfluidics (e.g. [23]) research and findings from this area may be usefully applied in this study.

The tribological properties of PDMS are now fairly well understood. Vorvolaskos and Chaudhury [24] investigated the effect of molecular weight and test temperature on friction in a pure sliding contact between a PDMS and metal surface. Bongaerts et al. [19] investigated the effect of surface roughness of PDMS on the lubricating properties of biopolymers and aqueous solutions. PDMS, like most elastomeric surfaces, is by nature hydrophobic but an oxidation treatment can be employed to create a hydrophilic surface. Hillborg et al. [25],[26] and Schneemilch et al. [27] investigated the wettability of PDMS before and after oxidation by several techniques and studied the effect of crosslink density on oxidation. de Vicente et al. [28] looked at the influence of surface modification of PDMS on its aqueous lubrication properties. However, there remains some debate over the suitability of PDMS as a model biosurface and instances of PDMS being tested under saliva conditions are few in number.

The second soft matrix to be considered here as a potential substrate to mimic the oral mucosa is agarose. Agarose, the agaropectin deficient fraction of agar derived from seaweed and consisting of β -1,3 linked α -galactose and α -1,4 linked 3,6-anhydro- α -L-galactose residues [29], is used to create a hydrogel-like matrix. The compliance

of agarose varies enormously depending on concentration, with Young's moduli ranging from ~1.5 kPa to ~3 MPa [30], [29], [31]. In addition to this, agarose has the ability to grow cells in suspension and has therefore been used in tissue culturing systems [31]. This combination of properties make agarose an attractive choice in biomedical research, for example, as a cartilage mimic [32], or as a phantom material for magnetic resonance elastography [33]. It is therefore surprising that agarose has been used in few tribological studies and seems to have been overlooked completely as an oral mimic. Fernández Farrés studied its frictional behaviour, but did so under glucose and glycerol lubrication rather than saliva [34]. Shewan et al also recently studied the lubrication performance of agarose, but as particles in suspensions rather than a substrate [35].

It can be concluded that it is important to be able to mimic the oral mucosa surface and various materials have been studied for this purpose. However, these have rarely been compared with actual biological materials (probably because of the difficulty in source, preserving and securing them) and almost never when lubricated by saliva. To address this, the current study characterises the friction and film thickness performance of polydimethyl siloxane (PDMS), agarose and porcine tongue, with the aim of assessing their suitability as an oral mimic for tribological testing. Particular attention is paid to the compliance and protein binding behaviours of these substrates.

2. TEST METHODS

2.1 Specimen preparation

PDMS specimens were moulded using a commercially available Sylgard 184 kit from Dow-Corning, containing a base and curing agent to produce a material with a Young's modulus 1.84 MPa at 23°C.

Agarose gel was produced by dissolving powdered agarose (Sigma-Aldrich, Poole, UK) into water at 1 or 2 % w/v. To aid dissolution the solution was heated to 90 °C then allowed to cool to a temperature below the coil-helix transition at around 35°C. At this point agarose forms a gel, consisting of an infinite three-dimensional network of fibre helices [29].

Prior to collection the subject refrained from food and drink for one hour. Resting whole mouth saliva (WMS) was collected from a single subject by drooling into a pre-weighed tube, kept on ice. After collection saliva was briefly centrifuged (3000 g for 3 mins) to remove sloughed cells and other debris.

Porcine tongue was procured and tested on the same day. Its underside was removed to produce a parallel slab. This specimen was then bonded onto a flat plate using cyanoacrylate adhesive and mounted in the friction rig.

2.2 Indentation and surface roughness measurements

The elastic modulus of each sample material was measured using an indentation test performed on a Mach 1 rig (Biomomentum Inc., Laval, Canada). This involved indenting the sample at 1 mm/s with a spherical indenter with radius 3.175 mm, during three repeat tests, while measuring the normal force and the vertical displacement. The normal force was measured using 1.5 N single-axis load cell with a resolution of 75 μ N and the vertical displacement was measured by the moving stage of the rig with a resolution of 0.1 μ m. A depth of penetration of 0.6 mm was used for agarose 1% w/v and 0.4 mm for each of the other samples. This was done in accordance with Van Dommelen et al.'s [36] suggestion that the sample thickness does not significantly affect the data if indentation depths are restricted to less than 10% of the sample thickness. Nevertheless a formulation that considers the finite thickness of the sample was used [37] to calculate Young's moduli. Contact mechanics equations were fitted to the data to give the Young's modulus, specifically,

$$\chi = \frac{a^2}{dR} \quad (1)$$

$$\kappa = \frac{P(1 - \nu)}{4aGd} \quad (2)$$

Where d is the displacement of the indenter, R is the radius of the indenter, a is the radius of the contact region, P the applied load, G the shear modulus, and ν the Poisson's ratio. A schematic of the test is provided in Figure 1.

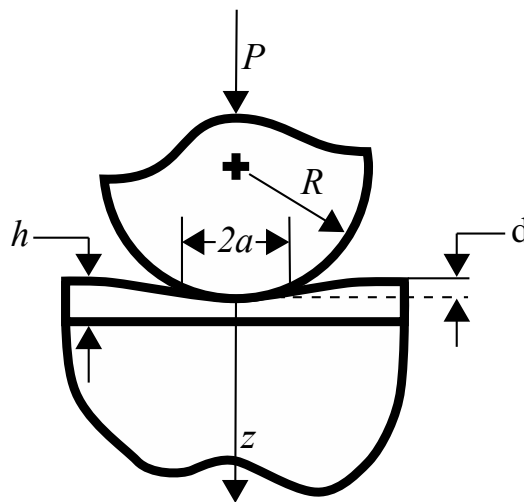


Figure. 1: Schematic diagram of indentation setup

The reaction force and the indenter displacement are recorded by the Mach-1 Motion Software and enter the above equations as P and d respectively. The Poisson's ratio is assumed to be equal to 0.5 (incompressible materials). The specimens' height h

and the indenter radius R are also known. The values of χ and κ are given in Table 2 in [37] for different values of a , h and ν . The radius of the contact region a is estimated during the curve fit with equation 1. Once the fitting algorithm converges, the Young's Modulus E is computed from the Shear Modulus G using the equation

$$E = 2G(1 + \nu) \quad (3)$$

The roughness of each of the specimens was measured three times (each at a different location) on the surface, using a Veeco optical profilometer.

2.3 Protein staining measurements

SDS-PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis) was used to assess the degree of binding of different proteins to the surface of the oral mimics. This immunoblotting technique targets proteins in a sample with specific dyes and measures their progression through a gel, due to an applied electric field. In this way, different proteins in a sample with different molecular weights are separated. Staining involved incubating for an hour at room temperature with whole mouth saliva from a single subject. Coomassie Brilliant Blue (CBB) was used stain of all proteins. In addition to this, Periodic Acid Schiff's (PAS) was used to stain for highly glycosylated proteins and specific antibody with sensitive chemiluminescent detection was used for the saliva protein muc7. Samples were removed from the surface of each of the oral mimic surfaces and tested in this way to investigate which proteins were present.

2.4 Friction measurements

A contact was produced by loading and sliding a 5 mm radius silica hemisphere against the compliant disc specimen, using a UMT2 (Universal Materials Tester), manufactured by CETR, (Campbell, USA). This equipment was operated in pin-on-disc mode, so that the PDMS specimen rotated, while the silica hemisphere was held stationary. The lower specimen was located on a rotating table, capable (with certain modifications), to run at speeds from 0.01 rpm up to ~ 4000 rpm. Friction force (F_x) and normal load (F_z) were measured using strain gauges, bonded to the housing above the stationary silica hemisphere specimen. Sensitive, low-load sensors were chosen for this purpose, with measurement ranges of ± 0.65 N and ± 6 N for F_x and F_z respectively. This experimental setup is shown in Figure 2 a. Friction data was recorded over a speed range from 0.002 to 0.35 ms⁻¹ with an applied load of 0.2 N.

2.5 Laser Induced Fluorescence measurements

The custom-built Laser Induced Fluorescence (LIF) microscope is shown by the photograph and schematic in Figure 2b. It comprises an LED light source, which produces a beam that is focussed through the transparent PDMS specimen onto the contact interface. For certain tests, the proteins in the lubricating saliva were tagged

with a dye, fluorescein isothiocyanate, (FITC) in order for them to fluoresce when excited by the LED. The emitted light is filtered and collected by a high-speed EMCCD camera. For film thicknesses greater than 200 nm, the recorded intensity of the fluorescence light emitted from the contact is proportional to the thickness of the liquid in the interface. This means that the images acquired by the camera represent maps showing the distribution of proteins in the contact. Further details of the fluorescence technique can be found in [38], [39].

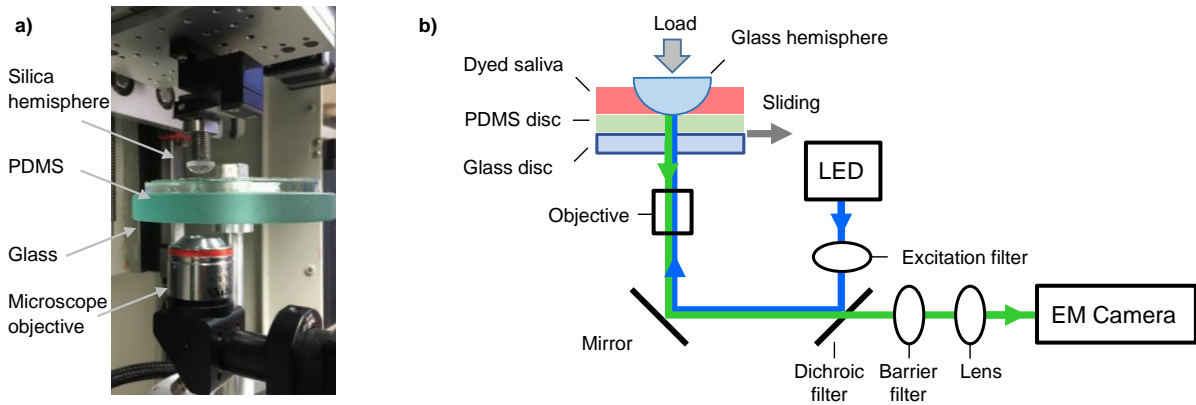


Figure. 2: Laser Induced Fluorescence setup, a) Photograph, b) Schematic diagram of indentation setup.

3. RESULTS

3.1 Indentation and roughness results

Figure 3 shows the force displacement curves for the four materials during the indentation tests using the Biomomentum Mach-1 rig. Equations 1-3 were applied to this data giving the Young's Modulus values shown in Table 1. As, expected the Young's Modulus of the porcine tongue at 3.5 kPa is lower than other measurements of biological tissue found in the literature – e.g. human skin: 25-101 kPa [40], human muscle: ~ 7 kPa [41]. These values were most closely mimicked by the agarose with a modulus 66 and 174 kPa for the 1 and 2% concentrations. The modulus of the PDMS was nearly two orders of magnitude higher than the biological sample.

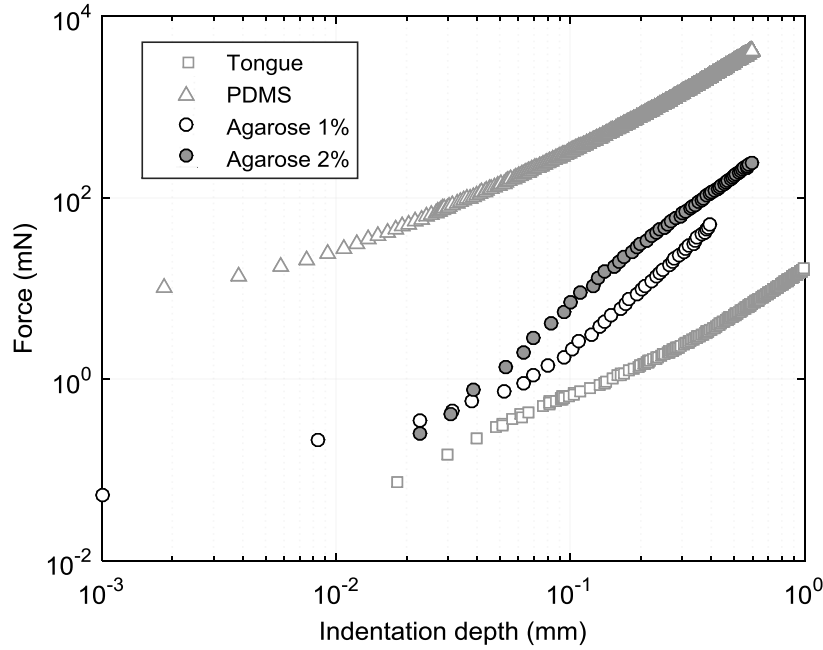


Figure. 3: Force-displacement curves for each material obtained during indentation.

Table 1: Young's modulus results for each test material in kPa.

Porine tongue	Agarose (1%)	Agarose (2%)	PDMS
3.46	66.4	174	2270

Table 2 shows the surface roughness measurements for each of the specimens, which are separated by approximately an order of magnitude (PDMS < Agar < Tongue). The effect this variation has on friction, however, is counteracted by the different stiffness which increases in the opposite sense (e.g. the asperities on the tongue surface are readily flattened). The range of values displayed for each measurement refers to the standard error, which is due to the variation over surface of the specimens, rather than any error in the measurement.

Table 2: Surface roughness results for each test material. Examples of the corresponding surface topographies are shown in the appendix.

	Roughness (nm)	
	Average (R_a)	RMS (R_q)
Tongue	5480 \pm 667	656 \pm 403
PDMS	10.1 \pm 0.16	13.1 \pm 0.23
Agar 1%	399 \pm 91	514 \pm 109
Agar 2%	325 \pm 14	420 \pm 18

3.2 Protein staining results

When incubated for an hour at room temperature with whole mouth saliva from a single subject, neither agarose nor PDMS bound significant amounts protein as shown by Coomassie Brilliant Blue (CBB) staining of all proteins as shown in Figure 4. Small amounts of amylase, the single most abundant protein in saliva, is the only protein apparent (identity based on apparent molecular weight). When the same gel was stained with Periodic Acid Schiff's (PAS), a stain for highly glycosylated proteins, small amounts of muc5b and muc7 were visible in the agarose gel but nothing in the sample eluted from PDMS. Immunoblotting for muc7 using a specific antibody with sensitive chemiluminescent detection again suggested agarose gel bound some mucins whereas PDMS did not. Incorporating potentially muco-adhesive agents such as chitosan and the lectin WGA (AWGL) into the agarose appeared to enhance protein, and mucin in particular, binding to the agarose.

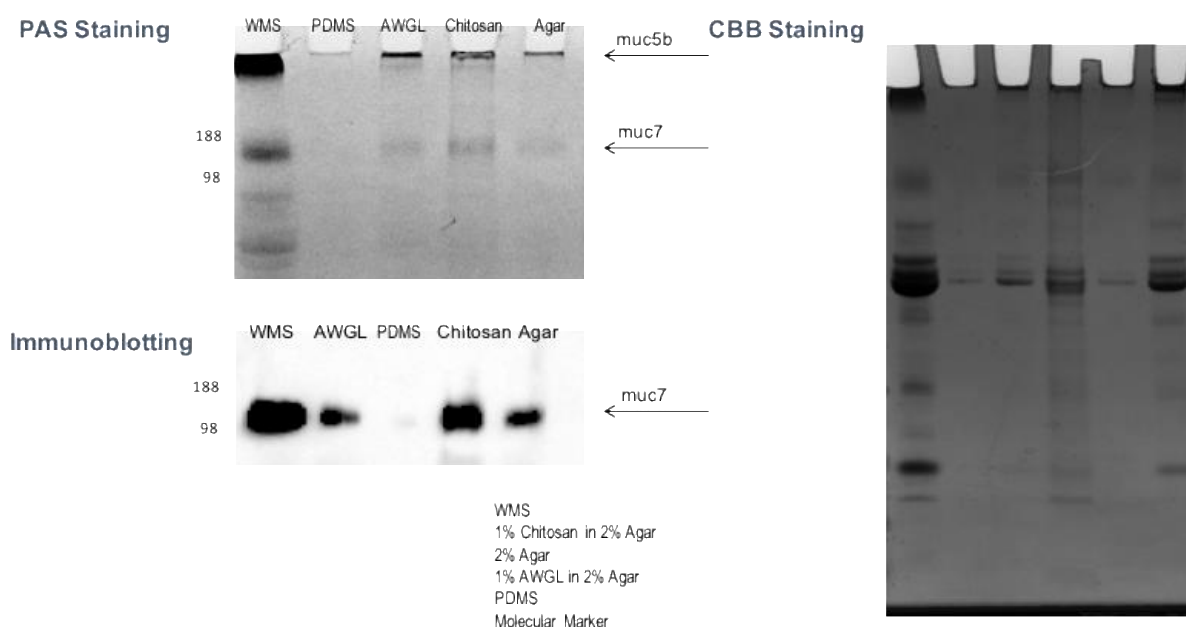


Figure. 4: Staining showing binding of proteins to each mimic surface. (note: the whole mouth saliva sample is labelled WMS)

3.3 Friction results

In this section, Stribeck curves have plotted the speed on the x-axis rather than the product of speed \times viscosity as is customary [15]. This is because the viscosity of saliva, being highly non-Newtonian, varies strongly as a function of shear rate [42] and is therefore not constant throughout each test. Another obstacle in assuming a single viscosity is the inhomogeneous and surface active nature of saliva means that it is not possible to assume whether it is the high viscosity proteins or just water molecules are entrained between the surfaces.

Figure 5 shows the variation in friction with sliding speed for the agarose-glass contact. Under unlubricated and water lubricated conditions, this substrate exhibits lower friction, due to the agarose being a hydrogel which releases water when compressed. When agarose was submerged in water it exhibits Stribeck curve behaviour with higher friction at low speeds which decreases rapidly with speed due to the formation of an elastohydrodynamic film. However, when lubricated with saliva, the friction behaviour is completely unchanged compared to pure water.

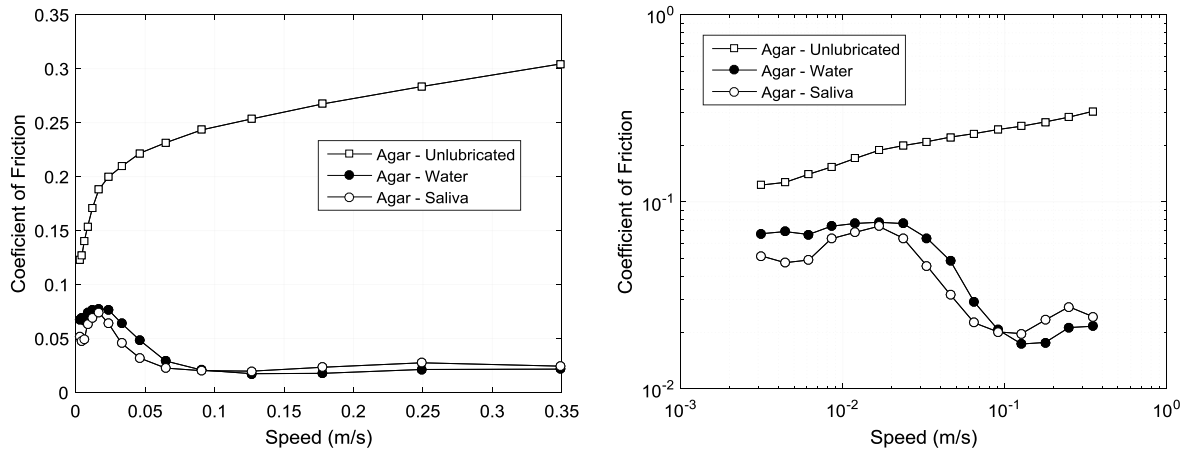


Figure. 5: Friction versus sliding speed for an agarose disc pressed against a stationary silica hemisphere with a force of 0.2 N. a) linear scale, b) log scale.

Figure 6 shows the variation in friction with sliding speed for the PDMS-glass contact under different conditions. When the contact is unlubricated, the coefficient of friction remains between 3 and 4, due to the strong adhesive interaction between the surfaces. The increase followed by a decrease in friction with sliding speed may be attributed to the viscoelastic properties of the elastomer (the friction that arises from the deformation of the PDMS varies as a function of speed due to its viscoelastic response).

At the lowest speed, the dry and water submerged friction values are similar, showing that no water is present between the surfaces even when submerged (*i.e.* no boundary film is formed). This is because the speed is insufficient entrainment of liquid to separate the surfaces and water molecules are not attracted to the PDMS surface. In contrast, when flooded with whole mouth saliva, very low friction (more than two orders of magnitude less than the dry case) is observed. These observations are in agreement with those of Stokes and co-workers [19].

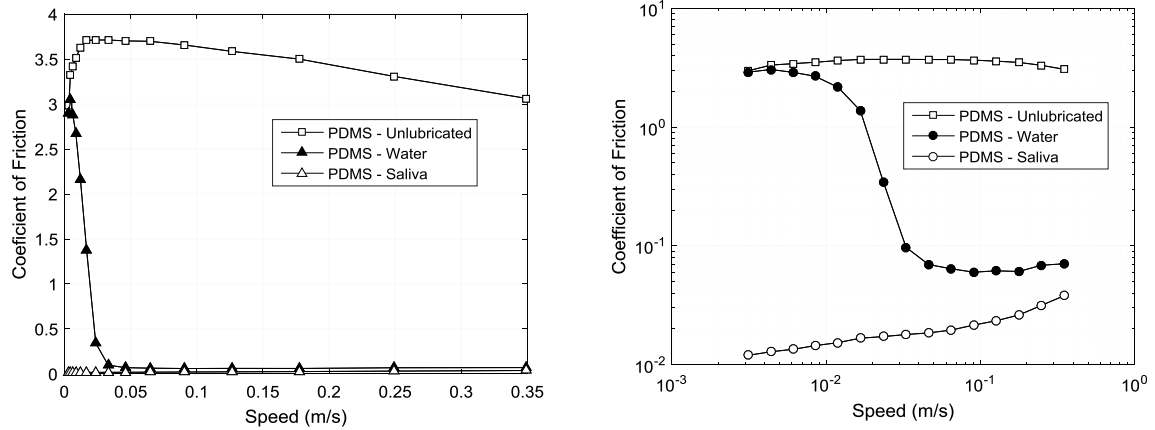


Figure. 6: Friction versus sliding speed for PDMS disc pressed against a stationary silica hemisphere with a force of 0.2 N. a) linear scale, b) log scale.

Figure 7 shows the friction versus speed behaviour for the porcine tongue sample. When lubricated with pure water, this sample shows high boundary friction which reduces with speed due to lubricant entrainment. In addition to this, the low speed boundary friction is significantly reduced when lubricated with saliva when compared to water. The shape of the dry, water and saliva lubricated curves are similar for PDMS and tongue, however there was a significant difference in terms of the magnitude of the friction.

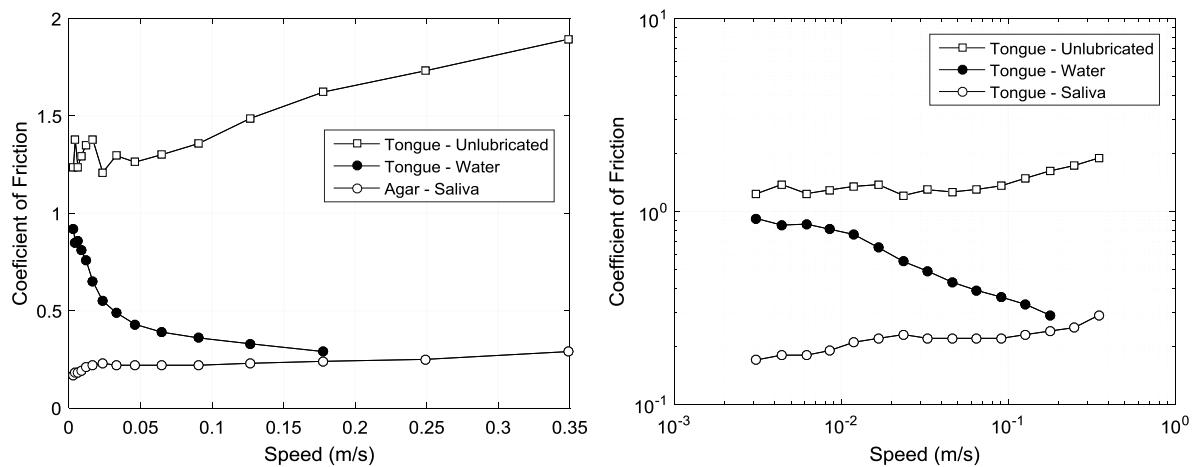


Figure. 7: Friction versus sliding speed for porcine tongue pressed against a stationary silica hemisphere with a force of 0.2 N. a) linear scale, b) log scale.

3.4 Laser Induced Fluorescence results

An advantage of PDMS over both the tongue and the agarose samples is that it is transparent, which enables imaging of the contact. To demonstrate this, the LIF microscopy results in Figure 8 show the build-up and flow of FITC-dyed saliva proteins within the contact during sliding. Images a-d in this figure are intensity maps of the contact showing the distribution of proteins (these are frames taken from the videos

provided as Supplementary Material). Here, bright colours represent high concentrations of proteins in the contact and the dark blue circular region is the pressurised contact area. Proteins agglomerations of varying morphologies are evident as they are entrained due the sliding motion from the inlet at the top of the figure to the outlet at the bottom. The figure also plots the variation in friction over time alongside a measure of the fluorescence intensity within the contact. The latter was obtained by counting the number of pixels within the contact with an intensity greater than the test average (using a Matlab program).

There is a clear correlation between the coefficient of friction and the presence of proteins within the contact zone. This is highlighted by the calculated cross correlation coefficient of 0.872 and the visible occurrence of peaks (shown by \wedge) in one single coinciding with troughs (shown by \vee) in the other signal, and vice versa.

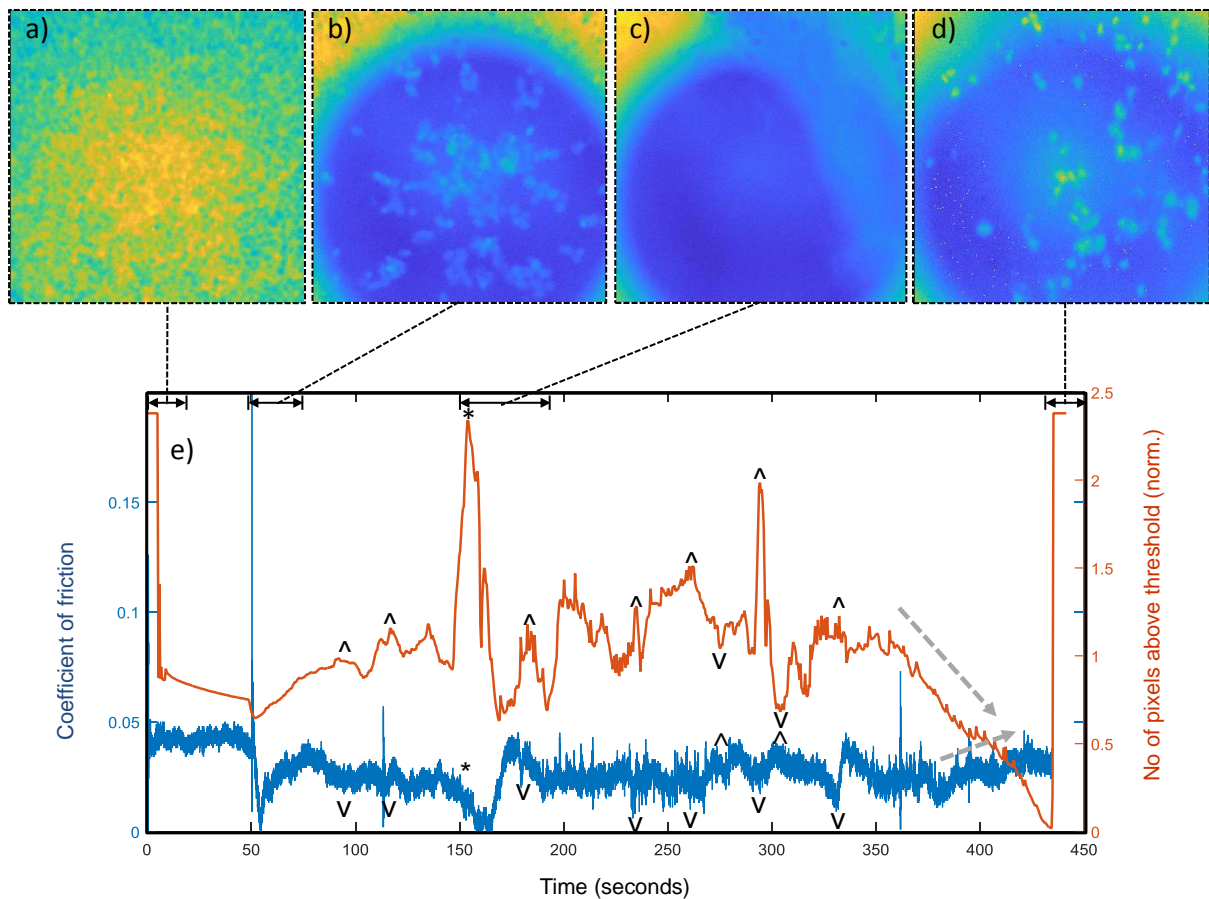


Figure. 8: Laser Induced Fluorescence results from a sliding test of silica hemisphere loaded against PDMS disc and lubricated with FITC dyed saliva. a) Intensity maps for unloaded contact, b) to d) Intensity maps during sliding, e) Variation of friction coefficient (blue) and fluorescence signal (orange), obtained by counting number of pixels with intensity greater than the test average. To highlight the correlation, example peaks are labelled with \wedge and example troughs are labelled with \vee . The arrows around 400 s highlight symmetrical trends in the two signals. Note: the step

changes in fluorescence observed at 5 and 440 s correspond to increase and decrease in in-contact proteins during the loading and unloading of the contact.

4. DISCUSSION

The stiffness of the tongue sample is far closer to that of the agarose than the PDMS. This means that that, for the agarose contact, the area and pressure match more closely those found in the mouth. Moreover, if this is considered in isolation, it suggests the boundary friction and hydrodynamic film thickness separating the surfaces for the agarose are more realistic. But it is important also to consider the mucosal pellicle for lubrication of oral surfaces by saliva and to implement this we added mucoadhesive components to agarose gels to enhance mucin binding. In some ways this appeared successful with greater amounts of all salivary proteins, including the two mucins (muc 5b and muc7), binding in greater amounts to the chitosan and WGA lectin containing agarose, shown by protein staining. However, there was little effect on the tribology when the mucoadhesive agarose was compared to agarose alone. Indeed, there was almost no difference between agarose lubricated by water or saliva. This suggests that this substrate is already being lubricated by the surface itself – probably water being expelled from the hydrogel under the pressure of the tribo-pairing. Furthermore, the interchangeability of the curves for water and saliva lubricated contacts in the full film regime, where friction is dominated by viscous drag, suggests high viscosity saliva proteins are not even being entrained into the contact at entrainment high speed.

The behaviour of PDMS showed much stronger protein interactions. When sliding at low speed (~ 0.1 mm/s) in the boundary regime (*i.e.* when there is insufficient hydrodynamic entrainment of liquid to separate the surfaces), the coefficient of friction for PDMS when lubricated by saliva is two orders of magnitude lower than when lubricated with pure water (~ 0.01 vs ~ 2). Since saliva is made up of 99.5% water and $<0.5\%$ protein molecules, this shows the proteins are highly effective surface active lubricating additives, which adhere to PDMS and oral surfaces to produce a lubricous low shear strength interface. More specifically, PDMS, like the tongue is hydrophobic [8] and due to its charged $-\text{O}-\text{Si}(\text{CH}_3)_2-$ group it attracts proteins indiscriminately [43] (in fact the adherence of biological proteins to PDMS is a problematic occurrence in biological lab-on-chip systems [43]). The viscosity difference between water and saliva (0.89 cP and ~ 5 cP [42]) is insufficient to explain this difference.

It could also be hypothesised that the elasticity of the bulk saliva may be responsible for the differences in the hydrodynamic/rheological response of the PDMS compared to water. However, at such low speeds elasticity should not play a role. Furthermore, as shown, the friction is strongly affected by the chemistry of sample surface, which would not be the case under full film hydrodynamic lubrication. Finally, as shown by Davies et al., the elasticity of resting saliva, as tested here, is significantly lower than that of acid stimulated saliva [44].

The shape of the dry, water and saliva lubricated curves for tongue are most similar to those of PDMS, which supports the latter's use as an oral mimic. However, there was a significant difference in terms of the magnitude of the friction. Under dry, unlubricated conditions, the PDMS shows a friction coefficient of around 3.5 in contrast to 1.5 for the tongue sample. When water is replaced with saliva, the PDMS friction reduces to ~0.02 while the tongue sample only falls to 0.25. This difference in friction coefficient magnitude between PDMS and tongue, under low speed conditions, when the surfaces are in contact, can be analysed as follows. As predicted by Schallamach [45] and Roberts [46], using Hertz theory, the coefficient of friction under dry/boundary lubrication conditions (*i.e.* when not liquid is separating the surfaces) is given by:

$$\mu = \pi S(9R/16E)^{2/3}W^{-1/3} \quad (4)$$

where R is the reduced radius, E is the elastic modulus, S is the interfacial shear stress and W is the load. This shows that higher friction coefficients arise in contacts between compliant materials, since these deform and produce a larger contact area to be sheared. Equation 4 can be used to calculate the shear stress within the contact, S , under boundary lubrication conditions since all other quantities are known, which gives values of 0.53 and 3.2 kPa for tongue and PDMS respectively. This suggests that, when lubricated by saliva, the lower friction of the PDMS surface arises due to its higher stiffness and smaller contact area, but the protein covered tongue surface is in fact more easily sheared. Another factor is the difference in roughness between the two samples. Under dry conditions, the lower roughness of the PDMS increases the real contact areas and hence adhesion, whereas under protein lubrication lower roughness aids the formation of a complete surface film.

The highly lubricious nature of the saliva proteins and their adherence to the PDMS surface are confirmed by the in-contact LIF results. In addition to demonstrating the effectiveness of this technique to study saliva protein entrainment, these results shed light on the details of this intermittent process. More specifically, the observed highly transient nature of the protein entrainment is similar to that demonstrated by Fan et al [47] who attributed the build-up and breakdown of proteins within the contact to the following inlet aggregation mechanism. Due to the contact geometry and flow path of the lubricant, proteins are transported into the contact inlet. Some of these proteins attach to the converging surfaces. Over time additional proteins become entangled with the surface protein branches, forming a larger protein mass in the inlet zone. A critical point is then reached where surface friction forces and lubricant hydrodynamic forces cause this protein mass to breakdown, allowing large agglomerate of proteins to be dragged into the contact zone. This can be observed in Figure 8, highlighted on the plot with a * symbol, where peak protein presence occurs with minima in friction coefficient.

The lubricating properties of saliva compared to water are assumed to relate to the salivary proteins such as mucins and statherin. Mucins contribute to the viscosity of

saliva which may aid the hydrodynamic mode of lubrication [19] whereas statherin, a small surface active protein is regarded as a boundary lubricant [48], [49] although it is entirely possible that other proteins also contribute to the lubricating properties.

5. CONCLUSIONS

From a surface chemistry point of view, PDMS is suitable at replicating the oral mucosa, since, like the tongue, it is hydrophobic [2] and its charged groups, which attract proteins [43]. This resulted in PDMS showing similar friction versus speed trends to the biological sample. Agarose on the other hand shows only a minor difference in friction when lubricated by saliva versus water. This is attributed to the hydrated agarose surface weakly adhering to the saliva proteins. The friction properties of agarose did not improve even after the agarose was treated with mucoadhesive components to enhance mucin binding

Although PDMS rubbers have similar hydrophobic qualities to a tongue, PDMS has an elastic modulus two orders of magnitude larger. Furthermore, even if the degree of cross linking is limited the modulus of PDMS reduces only to around 570 kPa [21] versus 3.4 kPa for tongue. This is significant shortcoming, since the stiffness of the sample affects both the boundary friction ($\mu \propto E^{-2/3}$ [45]) and the elastohydrodynamic film thickness ($h \propto E^{0.66}$ [28]). There is also considerable variation in roughness between the specimens tested, with agarose matching the tongue most closely. However, the effect this has on friction is limited due to the in-contact flattening of the rougher materials, which have lower stiffness.

An advantage of PDMS is that being transparent it allows in-contact imaging of saliva lubrication mechanisms. This was demonstrated using laser induced fluorescence and the resulting strong correlation (0.87) between friction and protein intensity signals confirms the lubricous boundary film forming ability of saliva proteins. Protein aggregation was shown to be highly transient in nature. The application of this technique to study the tribological interactions between saliva and foods and beverages in order to scientifically characterise mouthfeel attributes is the subject of ongoing research.

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APPENDIX – Surface topography measurements

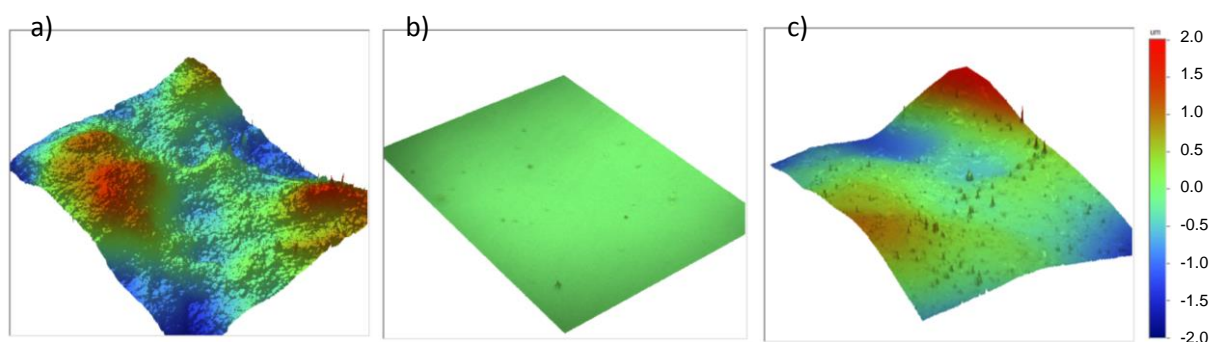


Figure. A1: Surface topographies of the three materials, measured using a Veeco optical profilometer, a) porcine tongue, b) PDMS, c) agrose.

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